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(54) Title: HUMANISED ANTIBODY AGAINST HEPATITIS

(57) Abstract

The invention relates to a humanised antibody capable of binding to a hepatitis antigen, in which the complementary determining regions of the variable domains are predominantly derived from a non-human antibody and the constant domains are predominantly derived from a human antibody.

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HUMANIZED ANTIBODY AGAINST HEPATITIS

The present invention relates to an antibody which binds to a hepatitis antigen, in particular hepatitis B surface antigen, to a pharmaceutical composition which contains the antibody, and to its use in the treatment and/or prophylaxis of hepatitis and hepatitis-related disorders.

Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat et al ("Sequences of proteins of immunological interest" US Dept. of Health and Human Services, US Government Printing Office, 1987).

A chimaeric antibody is described in WO 86/01533 and US Patent 4,816,567 in which the constant domains of a non-human antibody, such as a rat or mouse antibody, are replaced by human constant domains. A CDR-grafted antibody is described in EP-A-0239400 in which, in addition to the changes characteristic of a chimaeric antibody, the framework regions of the variable domains are replaced by human framework regions. Such humanised antibodies generally elicit less of an immune response, if any such

response, when administered to a human, compared to that elicited by a rat or mouse antibody. Humanised CAMPATH-1 antibody (Campath is a Trademark of The Wellcome Foundation Ltd.) is disclosed in EP-A-0328404.

Hepatitis viruses (which include type A, B, C [non-A, non B], D and E agents) cause significant morbidity and mortality in man. Not only do these agents produce acute infection of variable clinical severity but they also lead to or contribute to chronic liver disease terminating in cirrhosis of the liver, parenchymal liver failure and death. This acute and chronic hepatitis infection is a major medical problem in the United States and world-wide. It is also noteworthy that chronic hepatitis infection has been associated with primary hepatocellular carcinoma in endemic areas of the world.

It is estimated that 15% of the U.S. population have been infected with hepatitis B (HBV). In some African and Asian countries, the figure may be over 50% with 20% of the adult population remaining contagious as chronic HBV carriers.

HBV infection is transmitted by three general mechanisms: 1) by inoculation with infected blood or body fluids, either in large amounts (as in blood transfusions) or in small amounts (as in an accidental skinprick); 2) by close family or sexual contact; and 3) by infection during pregnancy, where the mother transmits the virus to her child.

Most HBV infections are subclinical, and recovery from both subclinical and clinical infections is usually complete. However, serious long term consequences occur in some cases: 1) about 5% of acute HBV infections result in chronic HBV infection, with the constant potential for infectivity to others and for serious, debilitating liver disease, and 2) past infection with HBV may be partly or even wholly responsible for the initiation of fulminant hepatitis, cirrhosis, and primary liver cancer. For example, the normal incidence of primary liver cancer is 1:100,000 but for chronic HBV sufferers the incidence is 1:300.

The widespread occurrence of HBV, together with its virulence and its association with chronic or lethal liver disease, constitutes a clinical problem of considerable importance. At constant risk are: 1) blood recipients, patients undergoing hemodialysis

or renal dialysis, and the institutionalized; 2) their families and 3) all health professionals (particularly nurses, surgeons and dentists).

The hepatitis viruses are known to express a number of antigens, for example hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antigen, hepatitis B Pre S1 and Pre S2 antigens, or antigens of hepatitis A virus (HAV), hepatitis C [non-A non-B], hepatitis D or hepatitis E virus(es). Antibodies have been raised against some of these antigens, for example EP0038642B discloses anti-HBV antibody secreted from a hybridoma cell line designated RF-HBs-1 and deposited with the collection Nationale de Cultures de Microorganismes (C.N.C.M.) at the Institut Pasteur, Paris under Accession Number 1-117.

According to the present invention there is provided a humanised antibody capable of binding to a hepatitis antigen, in which the complementarity determining regions of the variable domains are predominantly derived from a non-human antibody and the constant domains are predominantly derived from a human antibody. In particular an antibody according to the invention is capable of binding to a hepatitis B antigen such as hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antigen, hepatitis B Pre S1 and Pre S2 antigens. The invention is described hereinafter with particular reference to an antibody raised against a hepatitis B-surface antigen. This exemplification should not however been considered as limiting the invention.

An antibody according to the invention preferably has all or part of the structure of a natural antibody and may therefore be a complete antibody, a $(Fab')_2$ fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. Preferably, the constant domains contain an effector function, such as a complement-binding region. The antibody may be an IgG, such as IgG1, IgG2, IgG3 or IgG4; or IgM, IgA, IgE or IgD. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain.

Included within the scope of the invention are CDR-grafted antibodies, in which the framework regions of the variable domains are predominantly derived from a human antibody, and chimaeric antibodies, in which the framework regions of the variable domains are predominantly derived from the non-human antibody. CDR-grafted

antibodies are described in EP-A-0239400 and chimaeric antibodies are described in WO 86/01533 and US Patent 4,816,567, the contents of all three patent specifications being incorporated herein by reference.

It is preferred that the humanised antibody according to the present invention has a binding affinity to the antigen that is at least 30%, or even 50 or 70% of the affinity with which the non-human antibody, i.e. the antibody from which the CDRs are predominantly derived, binds to the same antigen. The binding affinity of the antibodies may be determined using standard assays known in the art in accordance with standard procedures.

The humanised antibody of the present invention is preferably capable of binding to a hepatitis B surface antigen and preferably contains CDRs with amino acid sequences that are identical to, or homologous with, the following sequences:

light chain: CDR1 (SEQ ID NOS: 3 and 4)
CDR2 (SEQ ID NOS: 5 and 6)
CDR3 (SEQ ID NOS: 7 and 8)

heavy chain: CDR1 (SEQ ID NOS: 11 and 12)
CDR2 (SEQ ID NOS: 13 and 14)
CDR3 (SEQ ID NOS: 15 and 16)

If the amino acid sequences of the CDRs are not identical to the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 of Seq ID Nos: 3 to 8 and Seq ID Nos: 11 to 16 respectively, then the sequences preferably have at least 60, 70, 80 or 90% homology with such light chain CDRs 1 to 3 and heavy chain CDRs 1 to 3. In homologous sequences, each CDR may include substitutions, insertions and/or deletions of amino acids. For example, there may be up to three amino acid substitutions, insertions and/or deletions in light chain CDR3 or heavy chain CDR3, up to four amino acid substitutions, insertions and/or deletions in light chain CDR1, and up to six amino acid substitutions, insertions and/or deletions in heavy chain CDR2. Most preferably the amino acid sequences of the CDRs are identical to the light chain CDRs 1 to 3 and heavy chain CDRs 1 to 3 of Seq ID Nos: 3 to 8 and Seq ID Nos: 11 to 16 respectively.

Preferably the framework of the variable region of the antibody heavy chain is substantially homologous to the corresponding framework of the human protein KOL (Schmidt et al, Hoppe-Seyler's Z. Physiol. Chem., 364 713-747, 1983). Homology in respect of the framework is generally 70% or more with respect to KOL, for example 80% or more or even 90% or more. A number of amino acid substitutions, insertions and/or deletions may therefore be present optionally to enhance binding of the antibody to the antigen. For example, the seventh residue of framework 4 is suitably Thr or Leu, preferably Leu. This residue is KOL residue 109 by Kabat et al, 1987. Other candidate framework changes that may be made to enhance binding include amino acid residues 27, 30, 48, 66, 67, 71, 91, 93 and 94. The amino acid numbering is according to Kabat et al.

The framework of the variable region of the antibody light chain is typically substantially homologous to the variable domain framework of the protein HSIGKVII (EMBL data base: Klobeck, H.G., EMBL data library submitted 7th April, 1986). There is a frameshift in this sequence at position 452. To rectify the reading frame, a deletion of base 452(T) may be made. Homology in respect of the framework is generally 80% or more with respect to HSIGKVII, for example 90% or more or even 95% or more. A number of amino acid substitutions, insertions and/or deletions may therefore be present, for example, at amino acid residue 71 according to the numbering of Kabat et al.

A humanised antibody is prepared according to the invention by a process which comprises maintaining a host transformed with DNA which encodes the light chain of the humanised antibody and with DNA which encodes the heavy chain of the humanised antibody under conditions such that each chain is expressed, and isolating the humanised antibody.

The DNA encoding the light chain and the DNA encoding the heavy chain may be on the same or different expression vectors.

Each chain of the antibody may be prepared by CDR replacement. The CDRs of a variable region of a light or heavy chain of a human antibody are replaced by sufficient

of the amino acid sequence of each CDR of the anti-hepatitis antibody such that the resulting antibody is capable of binding to the hepatitis antigen. The CDR-encoding regions of DNA encoding a hypervariable region of a human antibody chain are replaced by DNA encoding the desired CDRs. If appropriate, this altered DNA is linked to DNA encoding a constant domain for the antibody chain. The DNA is cloned into an expression vector. The expression vector is introduced into a compatible host cell which is cultured under such conditions that the antibody chain is expressed. Complementary antibody chains which are co-expressed in this way may then assemble to form the humanised antibody.

There are four general steps to humanise a monoclonal antibody by CDR-grafting. These are:

(1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains;

(2) designing the humanised antibody, i.e. deciding which antibody framework regions to use during the humanising process, and determining whether any additional amino acid changes may be required and effecting such changes;.

(3) the actual humanising methodologies/techniques; and

(4) the transfection and expression of the humanised antibody.

Step 1:

Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To humanise an antibody only the amino acid sequence of the antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody's variable domain amino acid sequence is from cloned cDNA encoding the heavy and light chain variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains. In the present instance by way of example, the nucleotide sequence and predicted amino acid sequence of the mouse RF-HBs-1 antibody chains are shown in SEQ ID NOS: 1 and 2 (light) and SEQ ID NOS: 9 and 10 (heavy).

Step 2:

Designing the humanised antibody

There are several factors to consider in deciding which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spatial orientation to recognize antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s).

A suitable human antibody variable domain sequence can be selected as follows:

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. The output of a suitable program is a list

of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if only human immunoglobulin sequences are included.

2. List the human antibody variable domain sequences and compare for homology. Primarily the comparison is performed on length of CDRs, except CDR3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent antibody CDR to one of the human subgroups as a first approximation of homology. Antibodies bearing CDRs of similar length are then compared for amino acid sequence homology, especially within the CDRs, but also in the surrounding framework regions. The human variable domain which is most homologous is chosen as the framework for humanisation.

Step 3:

The actual humanising methodologies/techniques

An antibody may be humanised by grafting the desired CDRs onto a human framework according to EP-A-0239400. A DNA sequence encoding the desired reshaped antibody can therefore be made beginning with the human DNA whose CDRs it is wished to reshape. The rodent variable domain amino acid sequence containing the desired CDRs is compared to that of the chosen human antibody variable domain sequence. The residues in the human variable domain are marked that need to be changed to the corresponding residue in the rodent to make the human variable region incorporate the rodent CDRs. There may also be residues that need substituting in, adding to or deleting from the human sequence.

Oligonucleotides are synthesized that can be used to mutagenize the human variable domain framework to contain the desired residues. Those oligonucleotides can be of

any convenient size. One is normally only limited in length by the capabilities of the particular synthesizer one has available. The method of oligonucleotide-directed in vitro mutagenesis is well known.

Alternatively, humanisation may be achieved using the recombinant polymerase chain reaction (PCR) methodology of UK Application No. 9022011.2. Using this methodology, a CDR may be spliced between the framework regions of a human antibody.

In general, the technique of UK Application No. 9022011.2 can be performed using a template comprising two human framework regions, AB and CD, and between them, the CDR which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers B and C each also contain, at their 5' ends, an additional sequence corresponding to all or at least part of the donor CDR sequence. Primers B and C overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed. Thus, the amplified regions AB and CD may undergo gene splicing by overlap extension to produce the humanised product in a single reaction.

Step 4:

The transfection and expression of the reshaped antibody

Following the mutagenesis reactions to reshape the antibody, the mutagenised DNAs can be linked to an appropriate DNA encoding a light or heavy chain constant region, cloned into an expression vector, and transfected into host cells, preferably mammalian cells. These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising:

(a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions

from a human antibody and the CDRs required for the humanised antibody of the invention;

(b) preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

(c) transforming a cell line with the first or both prepared vectors; and

(d) culturing said transformed cell line to produce said altered antibody.

Alternatively a single expression vector can be prepared followed by steps c) and d). Preferably the DNA sequence in step (a) encodes both the variable domain and the or each constant domain of the human antibody chain. The humanised antibody can be recovered and purified. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma e.g. YO or NSO cells, hybridoma, trioma or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

Antibodies according to the invention may be prepared using a recombinant expression system. One system is a mammalian expression system using Chinese hamster ovary (CHO) cells. These may be dihydrofolate reductase (dhfr) deficient and so dependent on thymidine and hypoxanthine for growth (PNAS 77 1980, 4216-4220). The parental dhfr$^-$CHO cell line is transfected with the antibody gene and dhfr gene which enables selection of CHO cell transformants of dhfr positive phenotype. Selection is carried out by culturing the colonies on media devoid of thymidine and hypoxanthine, the absence of which prevents untransformed cells from growing and transformed cells from resalvaging the folate pathway and thereby bypassing the selection system. These transformants usually express low levels of the product gene by virtue of co-integration of both transfected genes. The expression levels of the antibody gene may be increased by amplification using methotrexate (MTX). This drug is a direct inhibitor of the dhfr

enzyme and allows isolation of resistant colonies which amplify their dhfr gene copy number sufficiently to survive under these conditions. Since the dhfr and antibody genes are more closely linked in the original transformants, there is usually concommitant amplification, and therefore increased expression of the desired antibody gene.

Another expression system for use with CHO or myeloma cells is the glutamine synthetase (GS) amplification system described in WO87/04462. This system involved the transfection of a cell with a gene encoding the GS enzyme and the desired antibody gene. Cells are then selected which grow in glutamine free medium. These selected clones are then subjected to inhibition of the GS enzyme using methionine sulphoximine (Msx). The cells can be selected as they amplify the GS gene with concomitant amplification of the gene encoding the antibody.

Although the cell line used to produce the humanised antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. For single antibody chains, it is envisaged that E. coli - derived bacterial strains could be used. The antibody obtained is checked for functionality. If functionality is lost, it is necessary to return to step (2) and alter the framework of the antibody.

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, a humanised antibody may then be used therapeutically or in developing and performing assay procedures, immunofluorescent stainings, and the like (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The humanised anti-hepatitis antibodies of the present invention typically find use in the treatment and/or prophylaxis of hepatitis infections namely: hepatitis A, hepatitis B, hepatitis C (non A-non B) hepatitis D and hepatitis E; treatment of hepatitis in normal and hypogammaglobulinaemic individuals; suppression of growth of cells which express a hepatitis viral antigen on the surface, especially suppression of hepatocytes or hepatoma cells persistently infected with hepatitis B, one example is in the treatment of hepatocellular carcinoma (US 4714613): treatment of chronic hepatitis B sufferers undergoing liver transplantation due to liver failure (to prevent reinfection of the new liver); and treatment of accidentally infected individuals to avert full blown disease. An anti-HBV antibody may also serve to break tolerance in HBV carriers by binding to the HBsAg and making it more immunogenic.

The antibodies can also be used as separately administered compositions given in conjunction with antiviral chemotherapeutic or biological agents. Typically, the agents will include interferon, but numerous additional agents well-known to those skilled in the art may also be utilized.

An antibody of the present invention may form part of an immunotoxin. Immunotoxins are characterized by two components and are particularly useful for killing selected agents in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle", provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet", Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982).

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate,

adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). See, generally, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:335-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985).

The delivery component of the immunotoxin is a humanised antibody according to the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgA, IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The invention further provides a pharmaceutical composition comprising a pharmaceutially acceptable carrier or diluent and, as active ingredient, a humanised antibody according to the invention. The composition may comprise an immunotoxin according to the invention. The humanised antibody, immunotoxin and pharmaceutical compositions thereof of this invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously.

The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980).

The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The compositions containing the present human-like antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications, or sufficient to increase the immunogenicity of the hepatitis antigen to allow the patient to make a useful immune response against the virus. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the infection, but generally range from less than 0.2 to about 200 mg of antibody per dose, with dosages of from 0.5 to 25 mg per patient being more commonly used.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

For diagnostic purposes, the antibodies may either be labelled or unlabelled. Unlabelled antibodies can be used in combination with other labelled antibodies (second

antibodies) that are reactive with the humanised antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labelled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of a cellular activity or for the presence of a selected antigen. Thus, a humanised antibody of the present invention may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

The following Example illustrates the invention.

Cloning and sequencing of the RF-HBs-1 mouse anti-hepatitis B surface antigen heavy and light chains

Total RNA was isolated from 7×10^6 RF-HBs-1 expressing cells following the method of Chomczynski and Sacchi (Anal. Biochem. 162, 156-159, 1987), using 1ml of extraction solution per 1×10^7 cells. The resulting RNA pellet was redissolved in 50µl diethyl pyrocarbonate (DEPC)-treated distilled water, and spectrophotometrically determined to be at a concentration of 5µg/µl. Dynabeads Oligo $(dT)_{25}$ (Dynal) was

used to extract mRNA from 75μg total RNA employing the manufacturer's protocol. 0.2μg of mRNA was recovered.

cDNA was synthesised from the isolated mRNA and cloned into the plasmid pSPORT-1 using the Superscript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (BRL) following the method recommended by the manufacturer. Eschericia coli, Max Efficiency DH5a Competent Cells (BRL) were transformed with the resulting cDNA/pSPORT-1 ligation. Approximately 40,000 colonies were lifted onto Hybond-N nylon filters (Amersham) and lysed, denatured and fixed following the method of Buluwela et al., (Nucleic Acids Res. 17, 452, 1989). The filters were treated with proteinase K (50μg/ml) in 0.2 x SSC, 0.1% SDS at 55°C for 30 minutes and then excess debris removed with a tissue.

i) Heavy chain

An oligonucleotide as shown in SEQ ID NO: 17 complementary to a portion of rat gamma-CH1 (Constant Heavy domain 1) constant region (bases 496-515) was end-labelled and used to screen the filters for RF-HBs-1 heavy chain following the standard protocols relying on the homology between rat and mouse CH1 constant regions to identify the mouse clones. Approximately 30 potential positive colonies were dectected, and selected for further analysis. Plasmid DNA was prepared using the method of Del Sal et al (Nucleic Acids Res. 16, 9878, 1988) and 6 of the 30 contained inserts of the expected size for mouse immunoglobulin heavy chain cDNA. 6 Clones, were further selected, and the variable region sequenced in both directions by plasmid priming following the dideoxy chain termination method (Sanger et al (PNAS, USA, 74, 5463-5467, 1977)), according to the Sequenase kit (USB) protocol. Four clones had truncated heavy chains and 2 clones had full length heavy chains from which the variable region sequence was elucidated.
The sequence of the variable region is shown in SEQ ID NOS: 9 and 10.

ii) Light chain

A clone of the rat myeloma Y3-Ag 1.2.3 light chain (Crowe et al, Nucleic Acid Res. 17, 7992, 1989) was labelled with 32p dATP to screen the filters for the RF-HBs-1

light chain, following the manufacturer's protocol. Approximately 40 potential positive colonies were detected, and 18 selected for further analysis. Plasmid DNA was prepared as described above. Only RF-HBs-1 light chain was isolated (no clones expressed the MOPC 21 light chain which is synthesised by the mouse NS1 hybridoma fusion partner). One clone, containing the RF-HBs-1 light chain was chosen and sequenced as described for the heavy chain. The sequence of the variable region minus the terminal 7 amino acids in framework 4 is shown in SEQ ID NOS: 1 and 2.

Designing the chimaeric antibody

Using the selection procedure described in Step 2 above, the human variable domain frameworks of the KOL heavy chain (Kabat et al, 1987) and HSIGKVII light chain (EMBL data base; Klobeck, H.G. EMBL data library submitted 7th April, 1986) were chosen for the humanisation process. HSIGKVII Sequence from the EMBL database contains an extra T immediately 3' of CDR3 which must be removed to keep open reading frame.

Construction of the humanised heavy and light chain genes

The humanised heavy and light chains were constructed following the method of Lewis and Crowe (Gene 101, 297-302, 1991).

(i) Light Chain

Light chain oligonucleotide primers:

A_L: SEQ ID NO: 18:
B_L: SEQ ID NO: 19:
C_L: SEQ ID NO: 20:
D_L: SEQ ID NO: 21:
E_L: SEQ ID NO: 22:
F_L: SEQ ID NO: 23:
G_L: SEQ ID NO: 24:
H_L: SEQ ID NO: 25:

PCR reactions (Saiki et al. Science. 239, 487-491, 1988) were performed in a programmable heating block (Hybaid) using 20 rounds of temperature cycling (94°C for 1 minute 30 seconds, 50°C for 2 min, and 72°C for 3 min) followed by a final 10 min step at 72°C. 800ng of each primer, a specified amount of template, and 2.5 units of Taq polymerase (Perkin Elmer Cetus) were used in a final volume of 100µl with the reaction buffer as recommended by the manufacturer.

The initial template for the PCR was a previously constructed synthetic HSIGKVII light chain V region which had the CDR's replaced with CDRs from a rat Mab DX48.

Four primary PCR reactions were initially carried out, with 1.5µg of template per reaction, using the primer pairs A_L with B_L, C_L with D_L, E_L with F_L, and G_L with H_L respectively. The products of these PCR reactions, fragments AB_L, CD_L, EF_L and GH_L respectively, were purified using Prep-A-Gene (Bio-Rad) following the protocol recommended by the manufacturer. Fragments AB_L with CD_L, and EF_L with GH_L were combined using a quarter of each purified product, and subjected to recombinant PCR reactions with primers A_L plus D_L, and E_L plus H_L respectively. The products of these reactions, fragments AD_L and EH_L, were purified as above, and a seventh of each combined in a recombinant PCR reaction using primers A_L and H_L. The final humanised light chain recombinant PCR product, AH_L, was cloned into the HindIII site of pEE 12 (Celltech) following the method of Crowe et al. 1991, utilising the HindIII sites in primers A_L and H_L. Plasmid isolates were sequenced by the dideoxy chain termination method, and clones of the correct sequence chosen.

(ii) Heavy Chain

Heavy chain oligonucleotide primers:

A_H: SEQ ID NO: 26:
B_H: SEQ ID NO: 27:
C_H: SEQ ID NO: 28:
D_H: SEQ ID NO: 29:
E_H: SEQ ID NO: 30:
F_H: SEQ ID NO: 31:
G_H: SEQ ID NO: 32:

H_H: SEQ ID NO: 33:

The initial template for the PCR was humanised anti-CD4 heavy chain (on KOL framework; U.K. Patent Application No. 9020282.1; Gorman et al, Proc. Natl. Acad. Sci. USA 88, 1991) subsequently converted from genomic to cDNA context. The rodent CDR's were grafted on to the template following the recombinant PCR method as described above, but using oligonucleotide primers A_H to H_H and 0.24µg of template. Oligonucleotides A_H and H_H were designed with HindIII and EcoRI sites respectively to enable initial cloning of the humanised variable region, and a SpeI site was introduced into the KOL framework 4 (FR4) region of oligonucleotide G_H to facilitate subsequent cloning of the variable region with a suitable constant region of choice. The SpeI site altered the threonine residue at position 108 (Gorman S.D., Clark M.R., Routledge E.G., Cobbold, S.P. and Waldmann, H. PNAS 1991: 88. 4181-74185) (numbering according to Kabat et al, 1987) of the humanised anti-CD4 heavy chain template (proline in KOL) to a leucine residue (the majority of the six human heavy J-minigenes possess a leucine at this position; Kabat et al, 1987).

The humanised heavy chain variable region recombinant PCR product was cloned into HindIII/EcoRI-cut pUC-18 (BRL), and plasmid isolates of the correct sequence were chosen. The FR4 and c1 constant regions of the humanised anti-CD4 heavy chain were PCR cloned into pUC-18 (BRL) using oligonucleotide primers X_H (SEQ ID NO: 33) and Y_H (SEQ ID NO: 34). Primer X_H contains SpeI and HindIII sites, and Y_H an EcoRI site. The HindIII and EcoRI sites were used to clone the PCR product into pUC-18, and plasmid isolates of the correct sequence were selected. The complete heavy chain was subsequently reconstituted from the humanised variable region and c1 constant region clones using the engineered FR4 Spe1 site.

Transient Expression in COS cells

DNA encoding the humanised heavy and light chains were cloned into the vectors pEE6.hCMV and pEE12 respectively, see Stephens & Cockett [Nucleic Acids Res, 17, 7110, (1989)] Bebbington et al ., [Biotechnology, 10, 169, (1992)] (1992); and Bebbington and Hentschel [Glover Ed. DNA Cloning, Vol III, Academic Press, (1987)]. The vector pEE12 is a pBR322 - based vector containing the h-CMV-MEI

promoter and the hamster glutamine synthetase (GS) cDNA under control of the SV40 early region promoter. The vector pEE12 corresponds to pEE6 (see EP-A-0338841) with the GS cDNA expression cassette driven by the SV40 promoter transcribing in the same direction as the h-CMV-MEI promoter. Cells transfected with the vectors pEE6, hCMV and pEE12 are capable of growth in glutamine free medium because of the presence of the GS cDNA.

The recombinant plasmids (5μg of each) were transfected into $5x10^5$ COS-1 cells using the Transfectam reagent (Promega, Southampton, U.K.) under the conditions recommended by the manufacturer. Stock COS-1 cells (source ECACC, Porton Down, U.K.) were maintained in DMEM medium (Flow, Irvine, U.K.) supplemented with 10% foetal calf serum (APP, Dudley, U.K.). COS cell transfections were carried out in DMEM medium (Flow, Irvine, U.K.). Growth media from COS-1 cells four days post transfection were assayed by a sandwich ELISA assay using flexible microtitre plates (Falcon, Becton-Dickinson, Plymouth, U.K.) coated with polyclonal anti-human IgG (Sigma, Poole, U.K.) as capture antibody. The assay sample was added and detection performed with an anti-human IgG γ chain-specific peroxidase conjugate (Seralab, Crawley Down, U.K.) and orthophenylene dimine-HC1 (Sigma, Poole U.K.) as substrate.

The humanised antibody was shown to be expressed transiently in the COS cells by using the spent COS cell supernatant in the Wellcozyme anti-HBs assay, an amplified immunoassay for the detection of antibody to Hepatitis B surface antigen. Since the humanised antibody was shown to have retained antigen binding, stable NSO transfectants were generated.

Stable expression in NSO cells

A single expression vector for generating stable transfectants of NSO cells was generated by cloning the complete heavy chain expression cassette from pEE6 into the BamHI site of the pEE12 - light chain plasmid. Thus both heavy and light chain coding sequences are transcribed in the same direction from the same vector. 40μg of plasmid for transfection was linearised by digestion with SalI restriction enzyme that has a recognition sequence within the bacterial plasmid sequence. The linearised DNA was

precipitated from solution using ethanol, washed in 70% ethanol, dried and resuspended in sterile water.

Exponentially growing NSO cells (a Human myeloma cell line; see Jarvis, Methods in Enzymology, 73B, 3 (1981); source ECACC, Porton Down, U.K.) were maintained in non-selective DMEM medium (i.e. without glutamine and ferric nitrate but with sodium pyruvate at 110 mg/l (GIBCO/BRL, Paisley, U.K.) supplemented with 1X non-essential amino acids (Flow, Irvine, U.K.) 2mM glutamine (GIBCO) and 10% foetal calf serum (APP, Dudley, U.K.). NSO cells were centrifuged, washed and re-suspended in cold PBS, such that after the addition of the DNA the cells would be at a concentration of 10^7 cells/ml. The linearised plasmid DNA, 40µg, was added to 10^7 cells in an electroporation cuvette on ice. The cells and DNA were mixed gently so as to avoid generating bubbles and the mixture was left on ice for 5 minutes. The outside of the cuvette was wiped dry and two consecutive pulses at 1500V, 3mf were delivered using a Gene Pulser (Bio-Rad). The cuvette was returned to ice for 5 minutes.

Transfected cells were transferred to 96 well plates at densities of 3×10^5, 7.5×10^4 and 1.5×10^4 cells/ml in 50µl of non-selective medium and incubated at 37°C for 24 hours. Subsequently 100 µl of selective DMEM medium (i.e. without glutamine and ferric nitrate but with sodium pyruvate at 100 mg/l (GIBCO/BRL, Paisley, U.K.) supplemented with glutamate (60 mg/ml), asparagine (60 mg/ml; Sigma, Poole, U.K.), 1X non-essential amino acids, 7 mg/l of adenosine, cytidine, guanosine and uridine, 2.4 mg/l of thymidine (Sigma, Pool, U.K.) and 10% dialysed foetal calf serum (APP, Dudley U.K.)) was added to selected clones which had integrated the transfected plasmid. The plates were returned to the incubator and left until substantial cell death had occurred and discrete surviving colonies had appeared. Once colonies of glutamine-independent transfectants could be seen, wells with single colonies were selected and spent tissue culture supernatants were collected and assayed for human IgG secretion.

Wells with single colonies that were positive for IgG secretion were then expanded in culture using selective medium. The cells were distributed in 96 well plates at 10^4 cells/well in 100µl of medium and incubated overnight. 100µl of selective medium containing a concentration of L-methionine sulphoximine (MSX) was added. MSX is a

toxic glutamine analogue that allows for selection of vector amplification. Each 96-well plate had a different final concentration of MSX, ranging from 200μM down to 12.5μM. Individual colonies were isolated from each independent transfectant at the highest MSX concentration at which MSX resistance occurred. The colonies were expanded and antibody secretion rate (in $\mu g/10^6$ cells/day) was compared with the unamplified rate. Clones were obtained that expressed the humanised antibody at 0.5 to 9 $\mu g/10^6$ cells/day.

The humanised antibody was purified from spent tissue culture supernatant by affinity chromatography over a Superose protein-G column (Pharmacia) and used in the Wellcozyme Immunoassay.

Results of Wellcozyme immunoassay.

SAMPLE	A_{492}
Tissue culture negative control	0.147
Mouse MAB RFHBs-1 supernatant	0.167
Mouse MAB RFHBs-1 supernatant x10	0.208
Pool of humanised supernatants	0.193
Humanised clone F9 supernatant	0.270
Humanised clone HD10 supernatant	0.210

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE WELLCOME FOUNDATION LTD. OR JAMES SCOTT CROWE

(ii) TITLE OF INVENTION: ANTIVIRAL ANTIBODY

(iii) NUMBER OF SEQUENCES: 33

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: THE WELLCOME FOUNDATION LTD
(B) STREET: LANGLEY COURT
(C) CITY: BECKENHAM
(D) STATE: KENT
(E) COUNTRY: ENGLAND
(F) ZIP: BR33BS

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9202796.0 (PRIORITY)
(B) FILING DATE: 11-FEB-1992 (PRIORITY)
(C) CLASSIFICATION: -

(vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: STOTT, MR. MICHAEL JOHN

(B) REGISTRATION NUMBER: -

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 314 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..314

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAT GTT TTG ATG ACC CAA ATT CCA CTC TCC CTG CCT GTC AGT CTT GGA
Asp Val Leu Met Thr Gln Ile Pro Leu Ser Leu Pro Val Ser Leu Gly
 1               5                  10                  15

GAT CAG GCC TCC ATC TCT TGC ACA TCT AGT CAG AGT GTT GTC CAT AGT
Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Ser Val Val His Ser
            20                  25                  30

AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA CCA GGC CAG TCT
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
```

CCA AAG CTC CTG ATC TAC AAA GTC TCC AGC CGA TTT TCT GAG GTC CCA
Pro Lys Leu Leu Ile Tyr Lys Val Ser Ser Arg Phe Ser Glu Val Pro
50 55 60

GAC AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA CTC GAG ATC
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Glu Ile
65 70 75 80

AGT AGA GTG GAG GCT GAG GTA CTG GGA GTT TAT TAC TGC TTT CAA GGT
Ser Arg Val Glu Ala Glu Val Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

TCA CAT GTT CCG TAC ACG TTC GGA GG
Ser His Val Pro Tyr Thr Phe Gly
100

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Val Leu Met Thr Gln Ile Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Ser Val Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Ser Arg Phe Ser Glu Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Glu Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Val Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly
100

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..48

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACA TCT AGT CAG AGT GTT GTC CAT AGT AAT GGA AAC ACC TAT TTA GAA
Thr Ser Ser Gln Ser Val Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Thr Ser Ser Gln Ser Val Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAA	GTC	TCC	AGC	CGA	TTT	TCT
Lys	Val	Ser	Ser	Arg	Phe	Ser
1				5		

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Lys	Val	Ser	Ser	Arg	Phe	Ser
1				5		

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTT	CAA	GGT	TCA	CAT	GTT	CCG	TAC	ACG
Phe	Gln	Gly	Ser	His	Val	Pro	Tyr	Thr
1				5				

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Phe	Gln	Gly	Ser	His	Val	Pro	Tyr	Thr
1				5				

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 357 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GAA GTG AAG CTG GTG GAA TCT GAG GGA GGC TTA GTG CAG CCT GGA AGT
Glu Val Lys Leu Val Glu Ser Glu Gly Gly Leu Val Gln Pro Gly Ser
 1               5                  10                  15

TCC ATG AAA CTC TCC TGC ACA GCC TCT GGA TTC ACT TTC AGT GAC TAT
Ser Met Lys Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30

TAC ATG GCT TGG GTC CGC CAG TTC CAG AAA AGG GTC TAC GAA TGG GTT
Tyr Met Ala Trp Val Arg Gln Phe Gln Lys Arg Val Tyr Glu Trp Val
        35                  40                  45

GCA AAC ATT AAT TTT GAT GGT AGT ACC ACC TAC TAT CTG GAC TCC TTG
Ala Asn Ile Asn Phe Asp Gly Ser Thr Thr Tyr Tyr Leu Asp Ser Leu
    50                  55                  60

AAG AGC CGT TTC ATC ATC TCG AGA GAC AAT GCA AAG AAC ACC CTA TAC
Lys Ser Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80
```

CTG CAA ATG AGC AGT CTG AAG TCT GAG GAT ACA GCC ACG TAT TAC TGT
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

GCA AGA GAT CCG GGC TAT GAT TAC GTC CTT GAC TAC TGG GGC CAA GGC
Ala Arg Asp Pro Gly Tyr Asp Tyr Val Leu Asp Tyr Trp Gly Gln Gly
100 105 110

ACC ACT CTC ACA GTC TCC TCA
Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Glu Val Lys Leu Val Glu Ser Glu Gly Gly Leu Val Gln Pro Gly Ser
1 5 10 15

Ser Met Lys Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ala Trp Val Arg Gln Phe Gln Lys Arg Val Tyr Glu Trp Val
35 40 45

Ala Asn Ile Asn Phe Asp Gly Ser Thr Thr Tyr Tyr Leu Asp Ser Leu
50 55 60

Lys Ser Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Gly Tyr Asp Tyr Val Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAC TAT TAC ATG GCT
Asp Tyr Tyr Met Ala
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Tyr Tyr Met Ala
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 1..51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAC ATT AAT TTT GAT GGT AGT ACC ACC TAC TAT CTG GAC TCC TTG AAG
Asn Ile Asn Phe Asp Gly Ser Thr Thr Tyr Tyr Leu Asp Ser Leu Lys
1 5 10 15

AGC
Ser

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asn Ile Asn Phe Asp Gly Ser Thr Thr Tyr Tyr Leu Asp Ser Leu Lys
1 5 10 15

Ser

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAT	CCG	GGC	TAT	GAT	TAC	GTC	CTT	GAC	TAC
Asp	Pro	Gly	Tyr	Asp	Tyr	Val	Leu	Asp	Tyr
1				5					10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Pro Gly Tyr Asp Tyr Val Leu Asp Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGTGGATAGA CAGATGGGGC

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GATCAAGCTT CTCTACAGTT ACTGAGCACA

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCCATTACTA TGGACAACAC TCTGACTAGA TGTGCAGGAG ATGGAGGC

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTCCATAGTA ATGGAAACAC CTATTTAGAA TGGTACCTGC AGAAG

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGAAAATCGG CTGGAGACTT TATAGATCAG GAGCTG

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AAAGTCTCCA GCCGATTTTC TGGGGTCCCT GACAGG

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGTGTACGGA ACATGTGAAC CTTGAAAGCA GTAATAAACC CC

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TTTCAAGGTT CACATGTTCC GTACACGTTC GGCGGAGGGA CC

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GATCAAGCTT CTAACACTCT CCCCTGTTGA

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATCAAGCTT TACAGTTACT CAGCACACAG

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGCCATGTAA TAGTCACTGA AGATGAATCC

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GACTATTACA TGGCTTGGGT CCGCCAGGCT

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CAGATAGTAG GTGGTACTAC CATCAAAATT AATGTTTGCG ACCCACTCCA G

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ACCACCTACT ATCTGGACTC CTTGAAGAGC CGATTCACTA TCTCC

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTAGTCAAGG ACGTAATCAT AGCCCGGATC TCTTGCACAG AAATA

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GATCCGGGCT ATGATTACGT CCTTGACTAC TGGGGCCAAG GGACACTAGT CACCGTCTCC

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TAGAGTCCTG AGGGAATTCG GACAGCCGGG AAGGTG

CLAIMS

1. A humanised antibody capable of binding to a hepatitis antigen, in which the complementarity determining regions of the variable domains are predominantly derived from a non-human antibody and the constant domains are predominantly derived from a human antibody.

2. A humanised antibody according to claim 1, in which the framework regions of the variable domains are predominantly derived from a human antibody.

3. A humanised antibody according to either of the preceding claims, in which the constant domains contain an effector function.

4. A humanised antibody according to any of the preceding claims, in which the antibody has a binding affinity to the antigen that is at least 30% of the affinity with which the non-human antibody binds to the same antigen.

5. A humanised antibody according to any of the preceding claims, in which the antibody is capable of binding to hepatitis B viral antigen.

6. A humanised antibody according to claim 5, in which the antibody is capable of binding to hepatitis B surface viral antigen.

7. A humanised antibody according to claim 6, in which the amino acid sequences of the complementarity determining regions are at least 60% homologous with the following sequences :

Light chain : CDR1 (SEQ ID NOS: 3 and 4)
CDR2 (SEQ ID NOS: 5 and 6)
CDR3 (SEQ ID NOS: 7 and 8)

Heavy chain : CDR1 (SEQ ID NOS: 11 and 12)
CDR2 (SEQ ID NOS: 13 and 14)
CDR3 (SEQ ID NOS: 15 and 16)

8. A humanised antibody according to any one of the preceding claims for use in the treatment and/or prophylaxis of hepatitis.

9. A humanised antibody according to any of the preceding claims for the treatment of hepatocellular carcinoma.

10. A pharmaceutical composition which comprises a humanised antibody according to any one of the preceding claims and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

Intrantional Application No PCT/GB 93/00267

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12P21/08; A61K39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12P ; A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	MOLECULAR IMMUNOLOGY vol. 27, no. 3, March 1990, OXFORD, GB pages 303 - 311 Y. LI ET AL. 'Construction, expression and characterization of a murine/human chimeric antibody with specificity for hepatitis B surface antigen.' see the whole document ---	1-10
Y	NATURE vol. 332, 24 March 1988, LONDON, GB pages 323 - 327 L. RIECHMANN ET AL. 'Reshaping human antibodies for therapy.' see the whole document ---	1-10
	-/--	

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
01 JUNE 1993	21 -06- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT FFICE	NOOIJ F.J.M.

Form PCT/ISA/210 (second sheet) (January 1989)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 263, no. 9, 25 March 1988, BALTIMORE MD, US pages 4059 - 4063 M. SHERMAN ET AL. 'Haloperidol binding to monoclonal antibodies. Hypervariable region amino acid sequence determination.' see figure 1 ---	1-10
A	BIOTECHNOLOGY vol. 7, no. 4, April 1989, NEW YORK, US pages 374 - 377 K. HARADA ET AL. 'Human-human hybridomas secreting hepatitis B virus-neutralizing antibodies.' see abstract -----	1-10